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(54) Diagnosis of malignant tumours by mRNA detection

(57) A method for the diagnosis or monitoring of cancer or a malignant tumour associated with a particular body tissue of which the normal cells have at least one specific active gene that expresses a particular mRNA and protein characteristic of that tissue is disclosed which comprises taking a sample of peripheral blood or other body fluid in which cells of said particular tissue are not normally present and testing said sample for the presence of such tissue cells using a detection technique that relies upon detecting the activity of a selected said tissue specific active gene as manifested through expression or transcription producing said particular mRNA. The detection technique described involves extracting the total cellular mRNA present from the sample, using reverse transcriptase to prepare complementary DNA (cDNA) from the mRNA extracted, and then carrying out a polymerase chain reaction (PCR) process with appropriate primers so as selectively to amplify the cDNA corresponding to the mRNA transcribed from the selected tissue specific active gene, followed by analysis of the PCR products using, for example, gel electrophoresis or other conventional means.

The claims were filed later than the filing date within the period prescribed by Rule 25(1) of the Patents Rules 1990.

This print takes account of replacement documents submitted after the date of filing to enable the application to comply with the formal requirements of the Patents Rules 1990.

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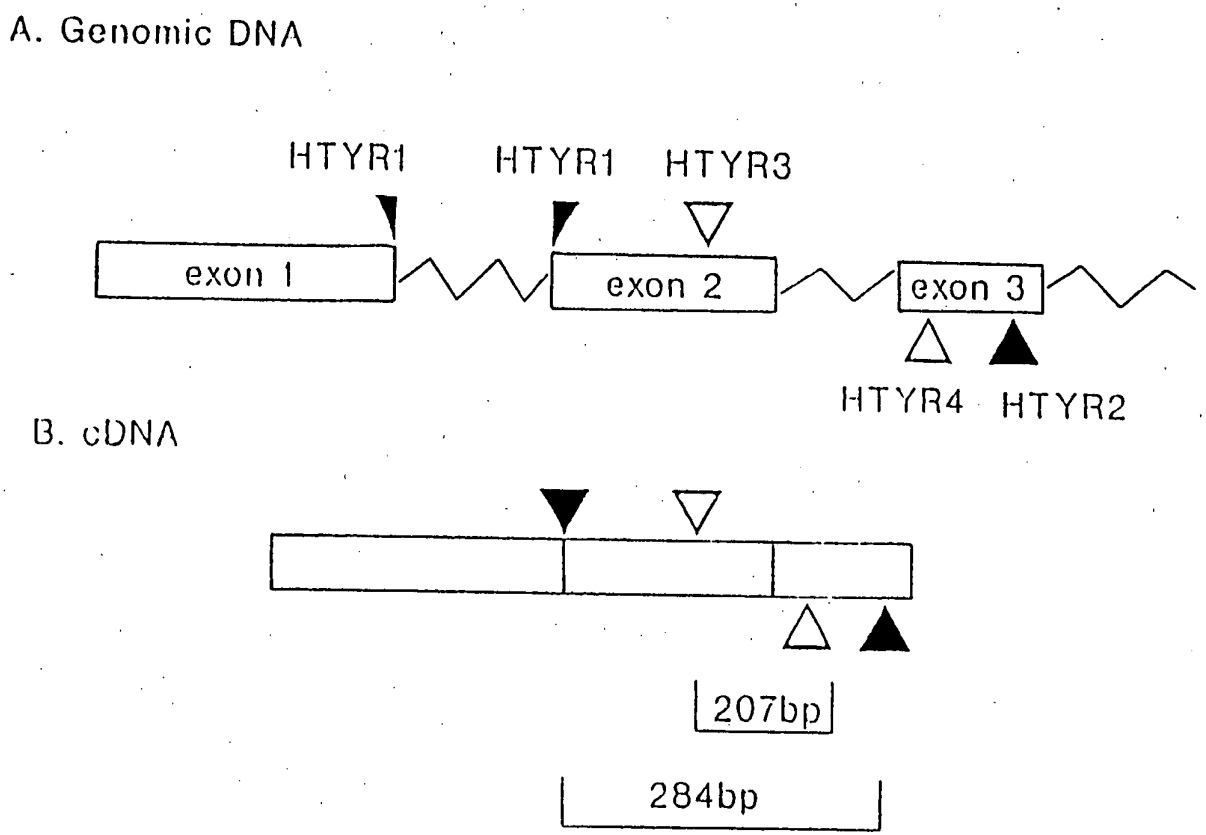
FIG.1.


A. Genomic DNA
HTYR1
HTYR1
HTYR3
exon 1
exon 2
exon 3
HTYR4
HTYR2
B. cDNA
207bp
284bp

DETECTION OF MALIGNANT TUMOURS

The present invention relates to the detection of malignant tumours and diagnosis of cancer in mammals. It can be of particular value for clinical use in connection with the diagnosis and/or monitoring of a variety of cancer conditions in human patients.

BACKGROUND

The development and growth of malignant tumours or cancers, at least insofar as many solid tumours in various specific tissues of the body are concerned, commonly results in the release of some of the cancerous cells from the developing tumour into the blood or other body fluids by which these cells are then transported to other parts of the body where they may become implanted and set up secondary tumours or metastases, thus leading to a general dissemination or spread of the original cancer that is responsible for production of the primary tumour. The process of metastases may commence at quite an early stage in the development and growth of the primary tumour, and in fact it is metastases, frequently haematogenous metastases produced by cancer tumour cells circulating in the peripheral blood, that determines the outcome of the disease for most patients.

Detection of such cancer cells in body fluids, particularly peripheral blood, is therefore a matter of

some importance, both from the point of view of diagnosing an original cancer and for monitoring and keeping under surveillance progress of the disease during therapy. However, such detection is not an easy matter because the number of such cancer or tumour cells circulating in such body fluids, particularly peripheral blood, is generally very small and they cannot therefore be distinguished and readily detected by routine microscopy. Techniques for their detection therefore need to be highly sensitive but must remain specific, and hitherto such methods as have been used have been dependent upon known abnormalities of the cells themselves arising from their cancerous condition.

It has for example been proposed by T.J. Moss and D.G. Sanders (Journal of Clinical Oncology, (1990), 8, 736-740) to detect neuroblastoma cells in circulating blood by means of an immunocytological analysis of blood samples involving the use of specific antibodies to certain cell surface antigens characteristic of the tumour cells concerned. However, although capable of high sensitivity this method is dependent upon the availability of the necessary specific antibodies for the particular tumour cells and is consequently of limited value for general use in relation to a range of different kinds of tumour cells.

It has also been proposed to detect particular

cancer cells in circulating blood by employing a technique involving the extraction of cellular mRNA from blood samples and the use of reverse transcriptase and a polymerase chain reaction (PCR) process to amplify complementary DNA (cDNA) corresponding to that mRNA whose transcription or expression is specifically related to an abnormal genetic defect characteristic of the cancer cells in question (see for example Sawyers et al, "Molecular relapse in chronic myelogenous leukemia patients after bone marrow transplantation detected by polymerase chain reaction", Proc. Nat. Acad. Sci. USA, (1990), 87, 563-567). However, this technique as previously disclosed for detecting tumour cells in the circulatory system has hitherto again been applicable only to cancers for which specific genetic abnormalities have been characterised, e.g. in respect of known translocations or mutations in the genomic DNA, but knowledge of such specific genetic abnormalities is not at present available for the majority of common solid cancers.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a method of more general applicability for detecting the presence of cancer or tumour cells in body fluids, especially peripheral blood, which is not dependent upon prior knowledge of specific abnormalities of the cells in question.

The method provided by the invention seeks specifically to detect in body fluids, such as peripheral blood, tumour cells from tissues whose cells are not normally present in such body fluids. By detecting the presence of a particular tissue cell in such body fluids where it should not normally be present, it has been realised that it can then be deduced that such tissue cell can with a high level of probability be regarded as being a cancer cell and indicative of a tumour in the tissue from which it is derived.

Thus, the invention provides broadly a method for the diagnosis or monitoring of cancer or a malignant tumour associated with a particular body tissue of which the normal cells have at least one specific active gene that expresses a particular mRNA uniquely characteristic of that tissue, said method comprising taking a sample of body fluid in which cells of said particular tissue are not normally present and testing said sample for the presence of such tissue cells using a detection technique that relies upon detecting the activity of a selected said tissue specific gene as manifested through expression or transcription producing said particular mRNA.

In carrying out the invention, the preferred detection technique involves extracting the total cellular mRNA present from a body fluid sample, preferably a sample of peripheral blood, using reverse transcriptase to

prepare complementary DNA (cDNA) from the mRNA extracted, and then carrying out a polymerase chain reaction (PCR) process with appropriate primers so as selectively to amplify the cDNA corresponding to the mRNA transcribed from the selected tissue specific gene, followed by analysis of the PCR products using, for example, gel electrophoresis or other conventional means.

It will be appreciated that the invention is especially applicable to cases where the sequence of the mRNA transcribed from the selected tissue specific gene is already known, or is at least partially known, such that suitable hybridizing primers can be provided to permit of the selective amplification of the complementary DNA by the polymerase chain reaction process.

Accordingly, from one aspect the invention more particularly provides a method for the diagnosis or monitoring of a cancer or malignant tumour associated with a particular body tissue in a mammal, said method comprising the steps of

(a) preparing an extract of total cellular mRNA in a sample of body fluid taken from said mammal;

(b) treating said extract of total cellular mRNA to prepare complementary DNA (cDNA) therefrom using reverse transcriptase;

(c) selectively amplifying said cDNA by carrying out a polymerase chain reaction (PCR) amplification

process using primers selected specifically to hybridize with a known unique DNA sequence of a gene that is generally active only in cells of said particular body tissue; and

(d) analysing the product of said PCR process to detect DNA corresponding to that of said particular body tissue active gene.

This method, in accordance with the invention, can be highly sensitive for detecting very low concentrations of the selected tissue cells and it does not depend upon any previous characterisation of cancer specific genetic abnormalities - on the contrary, it can be applied to any cancer for which tissue specific genes can be identified. The method may be useful in the diagnosis of either primary or metastatic cancers, in monitoring progress and determining the prognosis after surgical or medical therapy, and likewise in detecting residual disease following such therapy.

In practice, in clinical use, suitable control experiments or tests would be carried out to check the technique using, for example, cultured cells known to express the mRNA whose presence is being tested for and/or using alternative primers to amplify by the reverse transcriptase/PCR technique another protein mRNA known to be present in the body fluid sample concerned, e.g. the human β globin mRNA in samples of human blood. This

latter test is particularly desirable for checking the integrity of the extracted mRNA.

For practical use, all the basic essential materials and reagents required for carrying out the method may be assembled together in a self-contained kit. This will generally comprise at least the preselected primers for a particular tissue specific gene, Taq polmerase enzyme, and a mix of deoxynucleotides and buffers to provide the necessary reaction mixture for producing the cDNA and carrying out the selective PCR amplification. Thus, the invention also includes such kits for carrying out the method herein disclosed.

By way of example, the invention and the manner in which it may be carried out will be more particularly described by reference to some experimental investigations conducted using samples of peripheral blood from human patients suffering from malignant melanoma. This is a tumour with a wide range of metastatic sites which are mainly haematogenous. Normal melanocytes, or epithelial or mesenchyme cells, do not generally circulate in the peripheral blood, and one would not therefore expect them to be present in blood samples. Since only genes which are being actively expressed should be represented in cDNA derived from total mRNA in such blood samples, detection of genes whose activity is specific to these tissues should therefore provide evidence of the presence of these

tissue cells in the blood circulation and, hence, of an associated cancerous condition. For malignant melanoma there is a range of melanoma biosynthetic enzymes available for selection as tissue specific proteins, each representative of a particular mRNA uniquely characteristic of these tissue cells. However, for the purpose of the experimental investigations carried out, tyrosinase, which is an important enzyme in melanoma synthesis, was selected as a target tissue specific protein because its genomic organisation and cDNA sequence are known (see for example Y. Tomita et al (1989) "Human oculocutaneous albinism caused by single base insertion in the tyrosinase gene", Biochem. Biophys, Res. Commun. 164, 990-996 and B.S. Kwon et al (1987), "Isolation and sequence of a cDNA clone for human tyrosinase that maps at the mouse c-albino locus" Proc. Natl. Acad. Sci. USA 84, 7473-7477). As the tyrosinase gene contains a number of introns, for DNA amplification by PCR primers which are complementary to sequences represented on different exons of the gene were selected. PCR would be expected to amplify cDNA between the primers but genomic DNA would either not be amplified or would generate a PCR product of a much greater length.

DETAILS OF EXPERIMENTAL EXAMPLES

PCR Primer Sequences

From the published sequence for human tyrosinase cDNA, primer sequences were synthesised as follows:

HTYR1 (outer, sense)
5' TTGGCAGATT GTCTGTAGCC 3' (SEQ ID NO: 1)

HTYR2 (outer, anti-sense)
5' AGGCATTGTG CATGCTGCTT 3' (SEQ ID NO: 2)

HTYR3 (nested, sense)
5' GTCTTTATGC AATGGAACGC 3' (SEQ ID NO: 3)

HTYR4 (nested, anti-sense)
5' GCTATCCCAG TAAGTGGACT 3' (SEQ ID NO: 4)

The relative positions of these primer sequences with respect to human tyrosinase DNA are indicated in FIGURE 1 of the accompanying drawing which is a diagrammatic representation of the first three exons of the human tyrosinase gene and cDNA. The outer primers (represented by the solid triangles) produce a PCR fragment of 284 bp. The nested primers (represented by the hollow triangles) produce a PCR fragment of 207 bp. It will be seen that the HTYR1 sequence comprises the end of the first exon and start of the second exon. The long intervening sequences (introns) are not represented in the cDNA or PCR products so that the genomic DNA should not be amplified.

Human β-Globin primer sequences were devised from the published sequence as follows:-

Glo4 (sense)

5' ACCCAGAGGT TCTTTGAGTC 3' (SEQ ID NO: 5)

Glo5 (anti-sense)

5' TCTGATAGGC AGCCTGCACT 3' (SEQ ID NO: 6)

These β-Globin primers hybridize with sequences located either side of the 2nd intron of the human β-Globin gene and produce a fragment of 283 bp.

Cell Cultures

Four established human melanoma-derived cell lines were used in these studies. These were the melanotic SK-Mel-19 and SK-Mel-23, and the amelanotic SK-Mel-28 and M5 cell lines. All cultures were maintained on a 1:1 mixture of Dulbecco's Modified Eagle's Medium and RPMI 1640, supplemented with 5% fetal bovine serum (Sera-Lab). Cultures were transferred using trypsin (0.25%) and versene (5mM ethylene diaminotetracetate, EDTA).

RNA preparation, reverse transcription and PCR

2 to 10ml blood were spun at 2,500 rpm for 5 minutes, the plasma discarded and the sample stored at -70°C before preparing total RNA by the well-known guanidinium thiocyanate/caesium chloride method. RNA was prepared from approximately 10^7 cultured cells using the same method. 10µl of RNA was heated at 90°C for 4

minutes, cooled rapidly and diluted to 20µl containing final concentrations of; 1 x PCR Buffer (10mM Tris pH8.4, 50mM KCl, 100µg/ml gelatin), 1mM each dATP, dCTP, dGTP and dTTP, 8mM $MgCl_2$, 25pmoles HTYR2, 20U "RNAguard" (Pharmacia) and 4U MMLV Reverse Transcriptase (Pharmacia). After incubation at 37°C for 1 hour, half the sample (10µl) was diluted to 50µl containing final concentrations of 1 x PCR buffer, 200µM each dNTP, 1.6mM $MgCl_2$, 150pMol HTYR1 and HTYR2, 0.1% Triton X-100 and 1U Taq DNA polymerase (Promega). Each sample was overlaid with oil, heated at 95°C for 5 minutes and 30 cycles PCR carried out (cycle conditions:- 65 seconds at 95°C, 65 seconds at 55°C, 50 seconds at 72°C). For reamplification using the nested primers HTYR3 and HTYR4, 5µl of a 1:100 dilution were amplified in 25µl reaction volume for a further 30 cycles. In order to minimise contamination, all components of the reactions (except RNA and DNA) were prepared in a class 100 cabinet (Cleansphere, Gelman Sciences) and positive displacement pipettes were used. PCR products were analysed by electrophoresis on 2% agarose gels followed by ethidium bromide staining. Blood RNA integrity was checked by RT/PCR using primers for human β globin mRNA (Glo4 and Glo5).

The human cDNA clones Pmel34 (tyrosinase) and JW102 (β globin) were used to confirm that PCR with each set of primers gave bands of expected sizes. Using outer primers alone and 30 cycles of PCR, tyrosinase mRNA from the

equivalent of 50 cells or more was easily detected in the melanoma cell lines, SK-mel-19, SK-mel-23 and SK-mel-28. This level of detection was greatly improved by further amplification using nested printers and RNA equivalent to less than one cell could be detected (results not shown). No tyrosinase mRNA was detected in the cell line M5, one of the two amelanotic lines tested. However, when SK-Mel-23 cells were added to 10ml samples of normal blood before RNA preparation, the sensitivity of the technique was reduced and the minimum number of melanoma cells detected using other primers alone increased to 10^4. On the other hand, at least 10 SK-Mel-23 cells/10ml blood could be detected after reamplification with nested primers.

In order to determine the level of detection more accurately, individual SK-mel-19 or SK-mel-28 cells were micromanipulated under an inverted microscope using a fine, heat-drawn capillary and different numbers added to 2ml normal blood samples. As few as 4 SK-mel-19 cells ($1/5 \times 10^5$ WBC) and 1 SK28 cells ($1/2 \times 10^6$ WBC) gave positive results.

Blood samples from 7 patients with malignant melanoma (MM), 4 patients with other cancers (OC) and 4 normals were analysed in a blind trial. 4 out of 7 MM patients gave positive results and 4 out of 4 OC patients and 4 normals were negative (see Table 1 at the end of the present description).

In addition to using nested primers, further verification that the PCR product was indeed homologous to tyrosinase cDNA was obtained by restriction enzyme digestion. cDNA from patient MM3 was reamplified on a larger scale and digested with 3 restriction enzymes, each of which should cut once within the amplified sequence. All enzymes produced fragments of approximately the correct size. The small proportion of uncut DNA could be explained by errors introduced into the restriction sites by the Taq polymerase.

Overall, the experimental investigation described produced very encouraging results by giving positives in a majority of the small number of patients with metastatic melanoma so far tested, and carrying out the PCR amplification as described (30 cycles in first and second rounds) no false positives were found in cancer patients or normal patients. It should perhaps be noted, however, that increasing the number of cycles from 30 to 40 in the second round of PCR was found occasionally to give some false positives.

In the control spiking experiments, the sensitivity of the test was very high (up to 1 cell in 2 x 10^6 WBC). The difference in detection levels between different cell lines could reflect different transcription levels of the tyrosinase gene. Although a direct measurement of the number of melanoma cells circulating in the patients

cannot be obtained, the level of positive results and the absence of false positives is encouraging for adopting this testing method for clinical use, particularly as further technical developments may increase sensitivity without introducing errors. It is not clear if the false positives seen after 40 cycles in the second round of PCR was due to very low levels of contamination as reported by other workers using the PCR process, or if it is due to "illegitimate transcription" of the tyrosine gene.

The method herein described is rapid (2 to 3 days), has the advantage of avoiding the use of radioactive isotopes, and seems likely to not only have important applications for malignant melanoma, but also for many other cancers. First, the diagnosis of a primary cancer may be possible by the demonstration of inappropriate cells in the peripheral blood of patients and hence invasive diagnostic methods might be avoided. Detecting such cells in patients who have a widespread disease at presentation seems possible based on the experience with melanoma. Although as yet it is unknown whether the test would be useful in patients with apparently localised disease in whom peripheral blood tumour tissue cells may be few or absent, once a cancer diagnosis has been made, the detection method of the present invention may also be valuable in determining prognosis since it seems likely that the presence in peripheral blood of tumour tissue cells will increase at least the probability of

metastases. Only extensive evaluation will determine whether or not the presence in peripheral blood of tumour tissue cells is always ultimately associated with metastases or whether the process of implantation is sufficiently inefficient for some patients to survive with such cells in the circulation. Nonetheless, after therapy the continued presence of circulating cancer cells is likely to be associated with a poor outcome. If the primary therapy is surgical excision, it may be possible to identify patients for whom further systemic therapy is appropriate such as adjuvant chemotherapy in breast cancer. When the therapy in question is chemotherapy or biological therapy, it may be possible to identify patients for whom drug regimens should be changed or for whom treatment can be discontinued because of lack of effect, thus sparing unnecessary toxicity.

As will be appreciated, the method of the present invention may be of quite general application and, as previously indicated, can be readily extended to any cancer for which tissue specific genes can be identified.

TABLE 1

Detection of tyrosinase and β globin mRNAs in blood from normal (N), malignant melanoma (MM) and other cancer (OC) patients.

ND = test not done due to limited sample size.

PATIENT No.	SEX	AGE	DIAGNOSIS	SITES OF DISEASE	Tyr. mRNA	Glo. mRNA
MM1	M	34	Melanoma	Left groin nodes, right-supraciav node, Sternum	-	ND
MM2	M	47	Melanoma	Abdominal nodes, Peritoneum, Stomach	+	ND
MM3	F	52	Melanoma	Left groin & nodes, Subcutaneous tissue	+	ND
MM4	M	34	Melanoma	R. axilla, R. chest wall Brain	-	ND
MM5	M	65	Melanoma	R. foot, R. groin, R. legin transit mets	-	+
MM6	F	61	Melanoma	L. thigh, L. groin, Lung	+	+
MM7	M	35	Melanoma	Liver	+	+
OC1	M	64	Metastatic Colorectal Ca.	Liver	-	ND
OC2	M	59	Metastatic Gastric Ca.	Liver	-	+
OC3	F	48	Adv.T.C.C. Bladder	Pelvis	-	+
OC4	F	53	Metastatic Colorectal Ca.	Liver	-	+
N1	M	36	Normal		-	+
N2	F	30	Normal		-	+
N3	M	34	Normal		-	+
N4	F	39	Normal		-	+

SEQUENCE LISTING

SEQ ID NO: 1
SEQUENCE TYPE: Nucleotide
SEQUENCE LENGTH: 20 base pairs
STRANDEDNESS: single stranded
TOPOLOGY: Linear
MOLECULE TYPE: synthetic DNA digonucleotide
ANTI-SENSE: No

TTGGCAGATT GTCTGTAGCC 20

SEQ ID NO: 2
SEQUENCE TYPE: Nucleotide
SEQUENCE LENGTH: 20 base pairs
STRANDEDNESS: single stranded
TOPOLOGY: Linear
MOLECULE TYPE: synthetic DNA
ANTI-SENSE: Yes

AGGCATTGTG CATGCTGCTT 20

SEQ ID NO: 3
SEQUENCE TYPE: Nucleotide
SEQUENCE LENGTH: 20 base pairs
STRANDEDNESS: single stranded
TOPOLOGY: Linear
MOLECULE TYPE: synthetic DNA
ANTI-SENSE: No

GTCTTTATGC AATGGAACGC 20

SEQ ID NO: 4
SEQUENCE TYPE: Nucleotide
SEQUENCE LENGTH: 20 base pairs
STRANDEDNESS: single stranded
TOPOLOGY: Linear
MOLECULE TYPE: synthetic DNA
ANTI-SENSE: Yes

GCTATCCCAG TAAGTGGACT 20

SEQ ID NO: 5
SEQUENCE TYPE: Nucleotide
SEQUENCE LENGTH: 20 base pairs
STRANDEDNESS: single stranded
TOPOLOGY: Linear
MOLECULE TYPE: synthetic DNA
ANTI-SENSE: No

ACCCAGAGGT TCTTTGAGTC 20

SEQ ID NO: 6
SEQUENCE TYPE: Nucleotide
SEQUENCE LENGTH: 20 base pairs
STRANDEDNESS: single stranded
TOPOLOGY: Linear
MOLECULE TYPE: synthetic DNA
ANTI-SENSE: Yes

TCTGATAGGC AGCCTGCACT 20

CLAIMS

1. A method for the diagnosis or monitoring in mammals of cancer or a malignant tumour associated with a particular selected body tissue of which the normal cells are known to have at least one specific active gene that expresses a particular mRNA coding for a protein specific to that tissue, said method comprising taking a sample of body fluid in which cells of said particular selected tissue are not normally present in the absence of cancer or a malignant tumour in said tissue but which can be expected to contain cells of said tissue when cancer or a malignant tumour of the tissue has developed, and testing said sample for the presence of such tissue cells using a detection technique that relies upon detecting the activity of a selected said tissue specific active gene as manifested through expression or transcription producing said particular mRNA coding for the corresponding tissue specific protein.

2. A method as claimed in Claim 1 wherein the detection technique employed involves the steps of extracting the total cellular mRNA present from said body fluid sample, using reverse transcriptase to prepare complementary DNA (cDNA) from the mRNA extracted, and then carrying out a polymerase chain reaction (PCR) process with appropriate primers so as selectively to amplify any cDNA corresponding to the tissue specific protein mRNA

transcribed from the selected said tissue specific active gene, followed by analysis of the PCR products to determine whether such amplified cDNA is present.

3. A method for the diagnosis or monitoring of a cancer or malignant tumour associated with a particular body tissue in a mammal, said method comprising the steps of

(a) preparing an extract of total cellular mRNA contained in a sample of body fluid taken from said mammal, which body fluid is likely to contain cells of said tissue only when cancer or a malignant tumour of said tissue is present;

(b) treating said extract of total cellular mRNA to prepare complementary DNA (cDNA) therefrom using reverse transcriptase;

(c) selectively amplifying said cDNA by carrying out a polymerase chain reaction (PCR) amplification process using primers selected specifically to hybridize with and amplify a known unique DNA coding sequence of a gene which is generally active, independently of any cancerous condition, in cells of said particular body tissue, but not in any cells normally present in said body fluid, to express a particular tissue specific protein; and

(d) analysing the products of said PCR process to detect DNA corresponding to that of said particular body tissue active gene.

4. A method as claimed in Claim 2 or 3, wherein the analysis of the products of the PCR amplification process is carried out using gel electrophoresis.

5. A method as claimed in any of Claims 2 to 4, wherein at least one of the primers used in the PCR amplification process is selected so as to hybridize with a sequence of said tissue specific active gene that in the genomic DNA is interrupted by an intron.

6. A method as claimed in any of Claims 2 to 5, wherein the PCR amplification process is carried out using a pair of primers that hybridize with sequences of said tissue specific active gene that in the genomic DNA are located on opposite sides of an intron therein.

7. A method as claimed in any of Claims 2 to 6, wherein the PCR amplification process is performed in two stages, the second stage being a reamplification of the products of the first stage and being carried out using a pair of primers that are nested within the sequence of said tissue specific active gene that is amplified by the pair of primers used in the first stage.

8. A method as claimed in any of Claims 2 to 7, wherein an accompanying corresponding positive control experiment or test is also carried out using mDNA or cDNA known to be equivalent to that of the selected said tissue specific

active gene.

9. A method as claimed in any of the preceding claims wherein the body fluid sample used is a sample of peripheral blood.

10. A method as claimed in any of Claims 2 to 8, wherein the body fluid sample used is a sample of peripheral blood and a portion of the extract of total cellular mRNA or of the cDNA prepared therefrom is also subjected to a PCR amplification process using alternative primers selected to amplify mRNA, or corresponding cDNA, coding for another protein such as for example β globin, known to be expressed by normal cells in said blood sample, thereby providing a check on the integrity of the extracted mRNA.

11. A method as claimed in Claim 9 or 10 wherein the particular selected body tissue contains melanocytes subject to malignant melanoma.

12. A method as claimed in Claim 11 wherein the selected said tissue specific active gene is the tyrosinase gene.

13. A method for the diagnosis or monitoring of a cancer or malignant tumour associated with a particular body tissue in a mammal, substantially as herein described.

14. A kit for carrying out the method claimed in any of

the preceding claims, said kit comprising at least the preselected primers for use in the PCR process to hybridize to the cDNA derived from mRNA transcribed from a said particular tissue specific active gene, Taq polymerase enzyme, and a mix of deoxynucleotides and buffers to provide the necessary reaction mixture for producing the cDNA and carrying out the selective PCR amplification.

Patents Act 1977
Examiner's report to the Comptroller under Section 17 (The Search Report)

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Relevant Technical fields

(i) UK Cl (Edition K) G1B (BAC)

(ii) Int Cl (Edition 5) C12Q 1/68

Search Examiner
C SHERRINGTON

Databases (see over)
(i) UK Patent Office

(ii) ONLINE DATABASES: WPI

Date of Search
18 JANUARY 1993

Documents considered relevant following a search in respect of claims 1-14

Category (see over)	Identity of document and relevant passages		Relevant to claim(s)
X	WO 91/05064 A1	(THE UNITED STATES OF AMERICA, AS REPRESENTED BY THE SECRETARY U S DEPARTMENT OF COMMERCE) whole document, especially page 6, lines 23-30	1-3, 13, 14 (at least)
X	WO 90/09456 A1	(BALAZS, VIKTOR) whole document	1-3, 13, 14 (at least)
X	US 5057410	(CETUS CORPORATION) whole document, especially Examples; Claims 1-24	1-3, 13, 14 (at least)
A	Blood 1988, 72(6), 2063-2068 Detection of the Molecular Abnormality in Chronic Myeloid Leukemia by use of ...		1

Category	Identity of document and relevant passages	Relevant to claim(s.

Categories of documents

X: Document indicating lack of novelty or of inventive step.

Y: Document indicating lack of inventive step if combined with one or more other documents of the same category.

A: Document indicating technological background and/or state of the art.

P: Document published on or after the declared priority date but before the filing date of the present application.

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